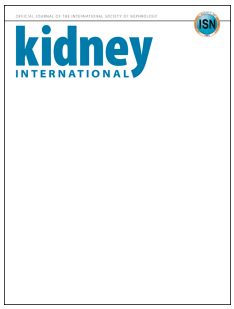
The eye, the kidney & cardiovascular disease: old concepts, better tools & new horizons



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Tariq E. Farrah, BSc MRCP, Baljean Dhillon, FRCS FRCPE FRCOphth, Pearse A. Keane, MSc FRCOphth MRCSI, David J. Webb, DSc FRCP, Neeraj Dhaun, PhD FRCP

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Kidney International

Invited review

The eye, the kidney & cardiovascular disease:

old concepts, better tools & new horizons

Tariq E Farrah BSc MRCP[1,2]
Baljean Dhillon FRCS FRCPE FRCOphth[3,4]
Pearse A Keane MSc FRCOphth MRCSI[5]
David J Webb DSc FRCP[1]
Neeraj Dhaun PhD FRCP[1,2]

[1]University/BHF Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK.
[2]Department of Renal Medicine, Royal Infirmary of Edinburgh, Edinburgh, UK
[3]Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK
[4]Princess Alexandra Eye Pavilion, Edinburgh, UK
[5]NIHR Biomedical Research Centre for Ophthalmology, Moorfields Eye Hospital, London, UK

Correspondence to: Dr Neeraj Dhaun
Centre for Cardiovascular Science
The Queen's Medical Research Institute
47 Little France Crescent, Edinburgh.
E-mail: bean.dhaun@ed.ac.uk

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Abstract

Chronic kidney disease (CKD) is common with hypertension and diabetes mellitus acting as major risk factors for its development. Cardiovascular disease is the leading cause of death worldwide and the most frequent endpoint of CKD. There is an urgent need for more precise methods to identify patients at risk of CKD and cardiovascular disease. Alterations in microvascular structure and function contribute to the development of hypertension, diabetes, CKD and their associated cardiovascular disease. Homology between the eye and kidney suggest that non-invasive imaging of the retinal vessels can detect these microvascular alterations to improve targeting of at-risk patients. Retinal vessel-derived metrics predict incident hypertension, diabetes, CKD and cardiovascular disease and add to current renal and cardiovascular risk stratification tools. The advent of optical coherence tomography (OCT) has transformed retinal imaging by capturing the chorioretinal microcirculation and its dependent tissue with near-histological resolution. In hypertension, diabetes and CKD, OCT has revealed vessel remodelling and chorioretinal thinning. Clinical and pre-clinical OCT have linked retinal microvascular pathology to circulating and histological markers of injury within the kidney. The advent of OCT angiography allows contrast-free visualisation of intra-retinal capillary networks to potentially detect early, incipient microvascular disease. Combining OCT's 'deep imaging' with the analytical power of deep learning represents the next frontier in defining what the eye can reveal about the kidney and broader cardiovascular health.

Keywords: chronic kidney disease, hypertension, imaging, microcirculation, ocular, proteinuria

Abbreviations

AVR – arteriole-to-venule ratio

BP – blood pressure

CKD – chronic kidney disease

CRAE – central retinal arteriolar equivalents

CRVE – central retinal venular equivalents

CVD – chronic kidney disease

Df – fractal dimension

eGFR – estimated glomerular filtration rate

ESRD – end stage renal disease

OCT – optical coherence tomography

OCT-A – optical coherence tomography angiography

GCL – ganglion cell layer

GBM – glomerular basement membrane

Introduction

Chronic kidney disease (CKD) affects ~10% of the world's population and its incidence is increasing.[1] Hypertension and diabetes mellitus are also common worldwide with an estimated prevalence of ~30% and ~10%, respectively; both are important risk factors for the development and progression of CKD.[2, 3] These systemic diseases are strongly associated with incident cardiovascular disease (CVD) and their inter-relationship contributes to CVD being the most common endpoint of CKD.[4] Current clinical tools lack precision to detect, stratify and track individual patients at increased risk of progressive CKD and CVD, and prior to end-organ damage. Thus, there is an urgent unmet need for simple, non-invasive methods to allow earlier identification and risk stratification of patients at increased risk of progressive end-organ injury and subsequent end-stage renal disease (ESRD) and CVD.

Microvessels (luminal diameter <300 μm) regulate tissue perfusion and contribute to systemic vascular resistance. This ability is closely linked to endothelial function. Several pathophysiological processes may contribute to *and* be a consequence of endothelial dysfunction, with downstream effects on microvessels (**Figure 1**).[5] Alterations in microvascular structure and function contribute to the development and progression of hypertension, diabetes, CKD and CVD.[5-7] Importantly, such changes precede the development of end-organ damage[8] and appear modifiable.[9] Moreover, microvascular dysfunction in peripheral beds mirrors dysfunction in visceral beds,[10, 11] providing a rationale for imaging accessible microvessels, such as those of the eye. Transparency of the ocular media allows direct visualisation of the microvasculature that may be affected by systemic diseases such as hypertension, diabetes and CKD. Here, we discuss the basis for the eye to act as a window to the kidney and evidence for the microcirculation of the eye to report risk of adverse renal and CVD outcomes.

The eye as a window to the kidney

The eye and kidney have several structural, developmental and organisational similarities that support the concept that ocular tissues might reflect renal disease (**Figure 2**).

Bruch's membrane and the glomerular basement membrane

Bruch's membrane divides the posterior pole of the eye into the retina (a laminated neurovascular structure) and choroid (an almost entirely vascular structure), collectively termed chorioretinal. Bruch's membrane and the glomerular basement membrane (GBM) both contain a network of α3, α4 and α5 type IV collagen chains.[12, 13] Thus, inherited or acquired diseases involving type IV collagen can affect both organs; the presence of co-existent nephropathy and retinopathy in Alport syndrome is a well-described example of this (**Supplementary figure 1**).[14, 15] Another example, anti-GBM disease is characterised by the development of immunoglobulin-G autoantibodies directed against the α3 chain, which are deposited on glomerular and alveolar basement membranes triggering a crescentic glomerulonephritis and pulmonary haemorrhage, respectively.[16] Similar linear immunoglobulin-G deposition in Bruch's membrane has been reported in patients with anti-GBM disease who developed concurrent choroidal ischaemia and retinal detachment.[17, 18]

The arrangement of the choroidal capillary (choriocapillaris) endothelium, Bruch's membrane and the retinal pigment epithelium mirrors that of the glomerular endothelium, GBM and podocyte (**Figure 2**). The pathological relevance of this homology is readily appreciated in membrano-proliferative glomerulonephritis type II in which electron dense deposits are found on the GBM and on Bruch's membrane.[19] Evidence of complement system dysregulation as a key driver of renal and retinal deposit formation in membrano-proliferative glomerulonephritis[20] and drusen deposition on Bruch's membrane in age-related

macular degeneration has extended the link between eye and kidney to include immune regulation.[21, 22]

Chorioretinal and renal microcirculations

Development and ultrastructure

The human retinal circulation develops predominantly by angiogenesis, where new vessels bud from pre-existing ones, to supply the inner two-thirds of the retina.[23] In the kidney, the peritubular capillaries and vasa recta populate the medulla and inner cortex in a similar manner.[24] In contrast, the choroidal and glomerular endothelium are reported to develop by vasculogenesis, where clusters of progenitor cells form islands of *de novo* vessels giving rise to the choriocapillaris and renal corpuscle respectively,[24, 25] although for the glomerulus this is debated.[26] The choriocapillaris endothelium has ~80 nm fenestrations allowing fluid exchange within the subretinal space.[27] The glomerular endothelium has similarly sized fenestrations that facilitate ultrafiltration into Bowman's capsule.[28]

Organisation and blood flow

The retinal and medullary circulations each receive <20% of the total ocular and renal blood flow, respectively, despite the high metabolic activity of the retinal photoreceptors and the medullary counter-current exchange system. Thus, both regions have a lower oxygen tension compared to their choroidal and cortical counterparts creating matched chorioretinal and corticomedullary oxygen gradients (**Figure 2**). The choroidal circulation receives ~80% of ocular blood flow and passively oxygenates key visual apparatus including the pigment epithelium and photoreceptors particularly within the avascular fovea.[25] This role demands a blood flow that is 4-fold higher *per* unit mass than the kidney and 10-fold higher than the brain,[25] indicating the importance of the choroid to global retinal health. Choroidal vascular

change may therefore predate the onset of overt retinopathy and, if detectable, might allow earlier identification of incipient disease.

Regulation of blood flow

All components of the renin-angiotensin-aldosterone system are widely expressed throughout the retinal and choroidal vascular networks (**Figure 2**).[29] Similar to effects in the kidney, angiotensin II acting *via* type I receptors leads to chorioretinal vasoconstriction[30] but may also modulate glial-pericyte-vasomotor signalling that maintains retinal neurovascular integrity.[31] Excessive renin-angiotensin-aldosterone system activation contributes to the pathogenesis of diabetic retinopathy and both diabetic and non-diabetic CKD.[32] Moreover, renin-angiotensin-aldosterone system inhibition in clinical trials prevents the development and progression of diabetic retinopathy and nephropathy, probably independently of effects on blood pressure (BP).[32]

Within the eye, endothelin-1 (ET-1) mediates vasoconstriction *via* endothelin-A (ET_A) receptors which are predominantly localised to choroidal and retinal vascular smooth muscle cells. In contrast, endothelin-B (ET_B) receptors appear confined to neuronal and glial structures.[33] Similarly in the kidney, ET_A receptors are localised to the vascular smooth muscle of glomeruli and vasa recta, whereas ET_B receptors are mainly localised to the collecting system (**Figure 2**). Selective ET_A receptor blockade in the eye increases retinal blood flow and reduces both retinal pericyte apoptosis and retinal thinning in a mouse model of type 2 diabetes.[34] These effects are mirrored in the kidney where selective ET_A blockade ameliorates intra-glomerular hypertension, podocytopathy and fibrosis to slow CKD progression.[35, 36] Autonomic innervation in the eye is limited to the choroidal circulation where sympathetic activation mediates choroidal vasoconstriction[25] in a similar manner to effects on intra-renal vessels. Thus, the choroidal microvasculature, rather than retinal

vessels, may more accurately reflect the renal microvasculature, particularly in diseases characterised by excessive sympathetic activation, such as CKD.[1]

Retinal imaging, the kidney and cardiovascular disease

Retinal photography

Qualitative retinopathy grading (for example microaneurysms, haemorrhages or focal arteriolar narrowing) and computer-assisted quantitative retinal vessel calibre analysis of digital fundus photographs have been the mainstay of retinal imaging for the last 20 years (**Supplementary figure 2**). As retinopathy reflects established end-organ damage detecting changes in retinal vessel calibre that precede this overt damage may allow earlier identification of at-risk patients.[37] The most established metrics are derived from arteriolar and venular widths from vessels close to the optic disc (**Table 1 & Supplementary figure 2)**. Novel indices of retinal vascular network geometry such as fractal dimensions (*Df*) can be derived from skeletonised vessel maps from retinal photographs (**Figure 3**). These indices identify suboptimal vascular branching patterns that may reflect and promote microvascular damage in systemic disease.[38, 39] The presence and severity of retinopathy, vessel calibre change and fractal deviations have been strongly linked to hypertension, diabetes mellitus and CKD as well as CVD endpoints.

The retinal circulation - CVD risk factors and outcomes

Hypertension

Retinal arteriolar narrowing is thought to reflect increased systemic vascular tone. Large cross-sectional studies demonstrate strong, independent associations between BP and generalised and focal arteriolar narrowing.[40] Longitudinal studies have shown that retinal arteriolar narrowing is associated with a ~2-fold increased risk of incident hypertension independent of age, sex, baseline BP and other CVD risk factors (**Supplementary table 1)**,

supporting the concept that retinal microvascular changes precede overt disease and are able to identify at-risk individuals. This paradigm has been challenged more recently by data suggesting a high prevalence of masked hypertension at the time of retinal imaging as detected by ambulatory BP monitoring.[41] Additionally, systolic BP and mean arterial pressure show an inverse linear relationship with *Df* in keeping with rarefaction.[42-44] This relationship holds true in young children with a normal BP (a population who should lack confounding pre-existing vascular risk factors) and is independent of retinal arteriolar calibre.[45]

Diabetes mellitus

Diabetic retinopathy is associated with systemic vascular complications likely reflecting widespread microvascular disease.[46] More so than in hypertension, retinal venular widening is prevalent in diabetes, correlates with severity of retinopathy and predicts progression to overt retinopathy suggesting a different pathophysiological basis for the change in vessel calibre.[47] Wider venules are seen in response to chronic hypoxia[48] and associate with endothelial dysfunction,[49] suggesting they reflect microvascular stress in response to metabolic derangement. In support of this concept, higher cholesterol, greater body mass index and worse glycaemic control link to wider retinal venules.[50-53] Moreover, wider venules and smaller AVR predict incident fasting hyperglycaemia and diabetes over 5 to 10 years, independent of fasting glucose, insulin levels, body mass index, family history or BP (**Supplementary table 2)**.[54] Finally, reduced *Df* in those with diabetes can predict incident neuropathy, nephropathy and progressive retinopathy independent of other risk factors for microvascular complications although the strength of these associations is modest.[55, 56]

CKD

Retinopathy (diabetic, hypertensive or otherwise) is more prevalent in patients with CKD, independent of standard CVD risk factors including diabetes and proteinuria.[57] Retinopathy

severity also shows a graded relationship with declining estimated glomerular filtration rate (eGFR) and its presence predicts future decline in renal function.[58, 59] Analysis of ~1000 patients from Chronic Renal Insufficiency Cohort with serial fundus photographs found that progressive retinopathy also tracks CKD progression in a subgroup of patients.[60] However, these initial associations were lost after adjusting for baseline risk factors for progression suggesting little added benefit of retinal metrics.[60] Both arteriolar narrowing and venular widening have been associated with prevalent CKD[61] but whether retinal vessel calibres predict incident or progressive CKD is not clear (**Table 2).** The Atherosclerosis Risk in Communities study examined retinal photographs of ~10,000 middle-aged patients and showed that those in the lowest AVR quintile had the greatest increase in serum creatinine over a six-year period; this held true after adjusting for baseline vascular risk factors.[59] Analysis of retinal images from ~4,500 patients without baseline CKD from the Multi-Ethnic Study of Atherosclerosis study found that narrower arterioles predicted the development of CKD in white patients alone.[65] However, other large, well designed studies have failed to find an independent association between any vascular calibre metric and CKD progression.[61, 64]

Albuminuria, an established marker of renal microvascular injury and an independent risk factor for CKD progression and incident CVD,[1] may better reflect retinal vascular changes. Cross-sectional studies have shown that a narrower CRAE is independently associated with greater albuminuria[71] and more severe glomerulopathy on histology in early diabetic CKD,[72] linking retinal and renal microvascular pathology. Additionally, baseline and subsequent arteriolar narrowing predicts worsening albuminuria and histological disease progression as well as decline in eGFR in both diabetic and non-diabetic CKD.[62, 66, 72] Importantly, using CRAE in conjunction with albuminuria allowed better stratification of individuals at increased risk of CKD progression than albuminuria alone.[66]

Studies exploring fractals in CKD are few and conflicting. A small study from Singapore (n=260) found a modest U-shaped relationship between *Df* and CKD, suggesting that *increased* vascular branching complexity, potentially due to neovascularisation as is often seen in diabetic retinopathy, is also indicative of increased risk.[73] A larger Malay study (n=3280) subsequently found lower *Df* to be strongly associated with lower eGFR and heavier proteinuria.[74] In contrast, large studies of both diabetics and non-diabetics found no relationship between either baseline or change in *Df* and the presence or progression of CKD (**Table 2**)[70, 75]

The lack of a consistent retinal vascular metric for the detection of incident and prevalent CKD may reflect the heterogeneity of the study populations, underlying aetiologies, metabolic abnormalities and treatments, such as immunosuppression and erythropoietin-stimulating agents. It may also suggest that retinal photography has limited sensitivity to reveal a reliable metric amongst these competing influences.

CVD outcomes

Retinal arteriolar narrowing, in contrast to venular widening, shows greater associations with atherothrombotic rather than metabolic CVD risk factors,[53] but both link to CVD outcomes. Large epidemiological studies and meta-analyses have shown that retinopathy, narrower arterioles and wider venules are common in patients with known ischaemic stroke disease.[76-78] Moreover, their presence predicts incident ischaemic stroke and stroke mortality, independent of other baseline CVD risk factors (**Supplementary table 3**).[79-81] These same metrics are also independent predictors of atherosclerotic coronary artery disease morbidity and mortality, which appear stronger for women than men (**Supplementary table 3**).[82, 83] A lower *Df* showed a linear relationship with worsening severity of coronary vessel stenosis in

~1,700 patients with ischaemic heart disease[84] and also associated with prevalent stroke.[85] In large prospective studies, a suboptimal *Df* conferred a 40% increased risk of stroke over a 5 year period[86] and a 50% increased risk of death from coronary artery disease over a 14-year period (**Supplementary table 3**).[87] These analyses included adjustment for standard CVD risk factors and retinal vessel calibres suggesting a better predictive ability of *Df*.[87]

The presence of retinal microvascular disease may also stratify patients at future risk of CVD as demonstrated by a recent study of >3,500 patients with microalbuminuria. Here, a wider CRVE predicted incident CVD over six years.[88] Furthermore, the presence of a single retinal microvascular abnormality, in conjunction with microalbuminuria, increased the risk of incident CVD 2-fold, and this rose to >6-fold if multiple vascular abnormalities were present.[88] Finally, and importantly, the use of retinal vascular metrics such as arteriolar narrowing and venular widening can provide added benefit over current atherosclerotic CVD risk prediction tools as demonstrated for ischaemic stroke[81] and coronary artery disease.[89]

These data provide robust evidence of the potential clinical utility of retinal imaging-based CVD risk assessment. Transition into clinical practice is still awaited and may have been hindered by inherent limitations (**Table 1**). The use of novel imaging modalities to target deeper vascular networks such as the choroidal circulation, which may reflect microvascular disease earlier and more accurately, might overcome some of these challenges. This is now possible through retinal optical coherence tomography (OCT).

Retinal OCT

Retinal OCT provides high-resolution, tomographic (cross-sectional) imaging of the eye with near histological detail.[90] The advent of retinal OCT has transformed clinical ophthalmology. In 2010, an estimated 16 million OCT scans were performed in the United States alone, more

than all other ocular imaging combined.[91] This rapid expansion has provided novel insights into the role of the chorioretinal microvasculature in the pathogenesis of age-related macular degeneration, diabetic retinopathy and glaucoma, eye diseases that have an increased prevalence in CKD.[61] The technical principles underpinning OCT are analogous to those of ultrasound but measure light reflections rather than the sound echoes (**Supplementary figure 3**). The measurement of small variations (interference) in light waves over short distances is made possible through the use of interferometry. In current generations of OCT, a Fourier transform analyses multiple interference signals simultaneously *via* a spectrometer (spectral domain OCT) or a tuneable laser (swept source OCT) resulting in extremely fast image acquisition.[90] Swept source OCT may also provide better tissue penetration than spectral domain OCT allowing better identification of deep structures like the choroid.[92]

The ultra-high resolution (typically 2-8μm) of OCT allows identification and automated segmentation of retinal layers providing measures of global and regional retinal thickness, volume and nerve fibre layer thickness allowing detection of retinal neurodegeneration. (**Table 3**, **Figure 4, Supplementary figure 4, videos 1 & 2**).[90] Importantly, OCT devices are capable of eye-tracking and image co-registration for accurate longitudinal imaging. Another key strength of OCT is the potential to image the previously inaccessible choroid, which is almost entirely composed of the blood vessels of the choroidal circulation (**Figure 4**). OCT-derived metrics of choroidal structure have been shown to be reproducible, correlate well with histology and may be surrogate measures for microvascular density with thinning suggestive of rarefaction.[93-95] In addition, the cross-sectional nature of the OCT image allows vessel wall/lumen analyses. OCT devices can also acquire *en-face* retinal images for vessel calibre assessment to complement choroidal imaging (**Supplementary figure 2).**[96] In short,

OCT allows comprehensive structural assessment of the entire chorioretinal circulation and its dependent tissue *in vivo* within a single platform.

The chorioretinal circulation: CVD risk factors and outcomes

Hypertension

We have used OCT to prospectively examine chorioretinal thickness in patients with established hypertension and age- and sex-matched healthy subjects.[97] We excluded patients with a history of eye disease, diabetes, or overt CVD. We found no differences in OCT metrics between these carefully matched groups. Two larger studies have produced contrasting results. The Beijing Eye study imaged ~3,000 adults and reported small increases in choroidal thickness with increasing diastolic BP but not systolic BP.[98] Paradoxically, the presence of hypertension was characterised by choroidal thinning. A subsequent cross-sectional study from Korea found that patients with hypertension (n=140) had significant retinal thinning in nearly all regions assessed compared to those without hypertension (n=687).[99] Notably the hypertensive group was older with a greater prevalence of CVD risk factors. More recently, a single centre study from Italy of 100 patients with hypertension found consistent retinal and choroidal thinning in hypertensives with co-existent CKD (defined by an eGFR <60 ml/min/1.73m^2 and/or moderate albuminuria) compared to hypertensives with preserved renal function.[100] These differences persisted following adjustments for age, antihypertensive use and glycaemia. The lack of a matched healthy control group in this otherwise well-designed study limits wider generalisability to CVD risk.

OCT also enables cross-sectional assessment of retinal vessels. Muraoka *et al* reported a greater arteriolar and venular wall thickness in 106 hypertensive patients compared to 132 patients without hypertension.[101] Given lumen size remained normal, their findings suggested outward vascular remodeling.

Interpreting the broader significance of these contrasting results in hypertension is difficult as different ethnic groups were studied, imaging devices varied, medication and comorbidity data were inconsistently reported, and those with diabetes were variably included. Large prospective studies that take such factors into account are needed to clarify any relationship and explore causality.

Diabetes mellitus

Studies using OCT to assess retinal thickness between patients with and without diabetes overall have shown significant thinning of the nerve fibre and ganglion cell layers even when retinopathy is absent or mild.[102, 103 104] Retinal ganglion cells are interneurons that transmit visual information from photoreceptor cells to the visual cortex via the optic nerve. Ganglion cell apoptosis is an early hallmark of retinal neurodegeneration which manifests as thinning of the ganglion cell layer (GCL) on OCT.[105] Such GCL thinning is not observable by standard retinal photography or scanning laser ophthalmoscopy.[105] Studies using OCT in types 1 and 2 diabetes, with no or minimal clinically observable retinopathy, have found selective GCL thinning compared to health.[102, 103] The degree of thinning correlates highly with duration of type 1 diabetes.[102] Studies of the choroid also show that patients with diabetes have thinner choroids compared to age- and sex-matched controls, even in the absence of retinopathy.[106, 107] Recent work suggests that greater reductions in choroidal thickness and choroidal vascular density occur with worsening retinopathy in keeping with progressive microvascular damage.[108] Whether the choroidal thinning contributes to, or indeed is a result of, retinal vascular disease needs further study. The choroid may therefore reflect early systemic vascular changes in diabetes such as glomerular hyperfiltration and hypertension.

Pre-dialysis CKD

We showed, for the first time, that patients with varying degrees of non-diabetic, pre-dialysis CKD exhibit ~5% retinal and ~25% choroidal thinning compared to both age- and sex-matched healthy subjects and patients with hypertension (**Figure 4**).[97] The highly vascular nature of the choroid suggests that thinning here is likely to represent changes in microvascular structure or function. Supporting this, we found that the choroid was thinner in those with a lower eGFR and greater proteinuria, both strongly associated with microvascular dysfunction.[109, 110] Also, those with a thinner choroid had higher circulating ET-1 and plasma asymmetric dimethylarginine (an endogenous inhibitor of nitric oxide synthesis) further supporting this association. Importantly, we linked chorioretinal thinning with renal histology, showing that the severity of glomerular injury reflected the degree of choroidal thinning. Recent data using different OCT platforms have confirmed our results. The previously discussed Italian study found that lower eGFR and greater microalbuminuria were associated with choroidal thinning using swept source OCT in 100 hypertensive patients.[100] Moreover, with respect to eGFR, this association was independent of age and other vascular risk factors.[100] These results are interesting as eGFR was preserved (~70 ml/min/1.73m^2) and proteinuria was low suggesting only modest microvascular damage. This contrasts with our work where CKD patients had moderate-to-severe renal disease (mean eGFR ~37 ml/min/1.73m^2, proteinuria equivalent to ~2 g/day).[97] Another recent study included patients with more advanced CKD and found a consistent relationship between lower eGFR, greater protein leak and a thinner choroid.[111] The differences in OCT metrics between CKD, hypertension and health, as well as their associations with CKD severity, provide initial evidence for the potential of OCT to identify and stratify individuals at increased CVD risk. Whether these metrics reflect systemic microvascular damage better than standard tools should be tested in future studies. Finally, the consistency of these findings across different

OCT devices using different technology and segmentation algorithms at least supports the fidelity of the relationship. Studies exploring whether this relationship can predict CKD outcomes are awaited.

ESRD

CVD risk is greatest in those with ESRD and on maintenance dialysis.[1] Haemodialysis is associated with repetitive subclinical myocardial injury, which has been linked to microvascular dysfunction that likely contributes to this risk.[112] A simple, non-invasive method of assessing microvascular disease in this very high-risk group would be useful. In keeping with our data, studies have shown that dialysis patients have global retinal thinning compared to healthy controls.[113] Additionally, a few small clinical studies in these patients have assessed how OCT metrics may be influenced by dialysis *per se* (**Table 4**). Collectively these studies suggest that the choroid, and the retina to a lesser extent, thins following dialysis with the greatest reductions seen patients with diabetic retinopathy. Whether thinning occurs because of changes in BP (altering chorioretinal perfusion), solute clearance, fluid removal or change in intraocular pressure is unclear and the studied cohorts are probably too small and/or heterogenous to assess these relationships in detail. Retinal nerve fibre layer thinning has been associated with neurodegenerative disease such as Alzheimer's disease and Parkinson's disease,[126] suggesting OCT can act as a window to the brain and cognition. Similar thinning has been found in haemodialysis patients[125] and supports recent data linking intradialytic cerebral hypoperfusion to progressive cognitive impairment.[127] There are no robust OCT studies in renal transplant recipients, but the available data suggest retinopathy and nerve fibre layer thinning are common.[128]

CVD outcomes

Studies linking OCT-derived metrics to prevalent CVD are beginning to emerge. Altinkaynak *et al,* studied 56 patients with heart failure with a reduced ejection fraction and found choroidal thinning of ~30% compared to age- and sex-matched healthy controls.[129] A thinner choroid was strongly associated with a worse ejection fraction in unadjusted analyses, which may reflect choroidal vasoconstriction secondary to reduced cardiac output.[129] No data on possible confounders such as renal function, diabetic status, CVD risk factors and concomitant drugs were reported.

Choroidal but not retinal thinning has been shown in patients with established coronary artery disease (defined by angiographic coronary artery stenosis, a positive stress test and/or previous coronary revascularisation or myocardial infarction) compared to health in small studies.[130, 131] However, limited reporting on possible confounding CVD risk factors, the inclusion patients with diabetes and a high CVD risk control group weaken these associations.[130, 131] A recent study using spectral domain OCT, examined a subgroup of 764 elderly patients (mean age 82 years, two-thirds female) from a French population-based study (>9,000 participants) and found no associations between subfoveal choroidal thickness and previous CVD, current CVD risk factors or estimated future CVD risk according to a clinical scoring tool.[132] A large amount of missing data, recall bias from patient-declared medical history and a single manual measure of choroidal thickness may have contributed to these negative results.

There are no data linking OCT-metrics to incident CVD to extend the relevance of the associations presented. These data are likely to appear soon and whether OCT-derived metrics can outperform photography-derived metrics for the prediction of CKD and CVD outcomes is an important test of their potential utility. An additional area that warrants exploration is the extent to which retinal OCT metrics are modifiable by interventions such as

lowering BP, reducing proteinuria or restoring kidney function. Such data might allow OCT-derived metrics to be developed from biomarkers into easily assessable surrogate endpoints for clinical trials.

OCT angiography (OCT-A)

Retinal OCT-A combines structural and functional imaging by analysing the changing variance in light speckle created by erythrocyte flow over multiple scans. This generates a contrast-free angiogram down to the capillary level and surrogate indices of perfusion (**Figure 5**).[90] Most OCT-A platforms have integrated software that automatically segments the OCT images alongside angiographic data to report global and regional vessel density for each retinal layer (**Supplementary figure 5; Supplementary videos 3 & 4)**. OCT-A images can be also be used to assess *Df* and the geometry of the foveal avascular zone (FAZ) with widening indicative of capillary drop out (**Figure 5)**. Visualisation of these terminal branches of the vascular tree may allow earlier, more precise detection of local and systemic microvascular disease. Small clinical studies of age-related macular degeneration[133] and diabetic retinopathy[134] have used OCT-A to demonstrate novel structural vessel pathology with an apparent reduction in perfusion. Limitations of OCT-A include a susceptibility to movement artefact degradation and a lack of true perfusion indices. Doppler OCT, which detects the frequency shift of back-scattered light from erythrocytes allowing determination of blood flow velocity alongside vessel dimensions, may overcome this latter issue.[90]

Hypertension, diabetes mellitus and CKD

Data supporting a potential role for OCT-A in systemic diseases are emerging. A study of Asian patients with hypertension found that those with poor BP control (assessed by 24h ambulatory monitoring) had a lower deep capillary plexus vessel density compared to those with optimal BP control.[111] These groups were well matched in terms of age, sex, CVD risk

factors and renal function (mean eGFR ~90 ml/min/1.73m^2) but the poorly controlled group had greater microalbuminuria.[111] In adjusted analyses suboptimal BP control, increasing BP and worsening eGFR were associated with worse capillary rarefaction.[111] An Italian study extended these findings by using OCT-A in 120 hypertensive patients with and without CKD, to report a lower vessel density in both superficial *and* deep capillary plexuses in those with CKD.[135] The inclusion of patients with more severe renal impairment and heavier proteinuria in this study[135] may explain the more extensive retinal vessel rarefaction seen compared to the Singapore study.[111] However, it is important to note different OCT-A devices were used by each centre.

In diabetes, greater FAZ area, lower retinal capillary density and reduced *Df* have been shown to predict progression of diabetic retinopathy.[136] A recent study using OCT-A has suggested a potential vascular basis for GCL thinning in diabetes.[137] Patients with no detectable retinopathy, with a short duration of diabetes (~8 years) and normal renal function, had significant GCL thinning that was independently associated with lower retinal capillary density and perfusion, suggesting a structural or functional vascular origin.[137] This hypothesis is countered by pre-clinical and clinical data suggesting that GCL thinning can occur without alterations in histologically-assessed capillary density.[138] In summary, GCL thinning appears early in diabetes, potentially prior to structural vascular changes but convincingly before overt target organ damage. The earliest functional changes in the diabetic kidney are glomerular hyperfiltration and hypertension.[139] and detecting this would be useful in guiding intervention and treatment efficacy. Whether GCL thinning or changes in GCL perfusion can act as an early marker of tissue dysfunction in diabetes such as glomerular hyperfiltration and hypertension warrants detailed further study. There are few data in diabetic CKD but a recent

study using OCT-A in 184 patients with type 2 diabetes suggested that a reduced retinal capillary density independently predicted co-existing CKD and its severity.[140]

Given the association with risk factors for CKD progression, data linking OCT-A to long term renal outcomes should soon emerge. However, identifying patients at risk of acute kidney injury (AKI) is also important as AKI confers an increased risk of future CKD[141] and CVD.[142] OCT-A-derived retinal vessel density was recently shown to predict the risk of contrast-induced AKI following angiography for acute coronary syndrome.[143] Moreover, the addition of OCT-A metrics to current contrast-induced AKI risk assessment tools improved prediction of AKI by ~10%.[143]

CVD outcomes

As with OCT, data linking OCT-A metrics to incident CVD are lacking. With respect to prevalent CVD, a study of 246 patients presenting with an acute coronary syndrome found that these patients had reduced inner retinal vessel density compared to a limited number of age- and sex-matched controls.[144] In addition, more severe rarefaction correlated with a greater CVD risk as defined by the *American Heart Association* and *Global Registry for Acute Coronary Events* scoring systems.[144]

Dynamic functional imaging

Laser doppler flowmetry and flicker response imaging can assess retinal vascular endothelial function in a dynamic manner. Studies examining laser flicker-induced vascular responses have shown impaired retinal endothelium-dependent vasodilatation in patients with CVD risk factors including albuminuria,[145] pre-diabetes,[146] early hypertension,[147] pre-eclampsia[148] and hypercholesterolaemia.[149] In addition, a recent study reported worsening retinal endothelial function (as measured by the degree of laser flicker-induced vasodilatation) between health,

those at risk of CVD and those with overt CVD,[150] supporting the potential to stratify patients. Limitations of these techniques include the need for mydriasis, longer acquisition time compared to other imaging techniques and assessment of retinal vessels alone.

Visions for the future

Pre-clinical OCT

Pre-clinical OCT allows simultaneous non-invasive longitudinal imaging of the eye in disease models and may provide novel insights into underlying mechanisms. We have used OCT to explore the links between the eye and kidney and shown that mice with hypertension alone had no choroidal thinning whereas mice with matched hypertension but with coexisting renal injury developed significant thinning.[97] This technology is being refined but has been used to perform detailed structural, functional and biochemical assessments in various models of retinopathy.[151]

Big data

The recognition of the potential power of OCT beyond eye disease is evidenced by its inclusion in the United Kingdom Biobank study between 2006 and 2010.[152] UK Biobank obtained OCT images from >67,000 participants (of whom >35,000 were healthy) along with socio-demographic, cognitive, CVD and renal risk measures. This could provide novel, robust epidemiological data to establish healthy ranges and generate evidence of OCT metrics as prognostic disease biomarkers. The expansion of OCT out of hospital settings and into the community is already in progress with the leading optometry chain in the United Kingdom announcing a roll out of OCT devices across all outlets.[153]

Deep learning

Machine learning involves programming computers to detect patterns in raw data, based on explicit parameters set by the operator. Such techniques have been utilised to automate classification of diabetic retinopathy from fundus photographs but can be intensive to engineer and supervise. Deep learning is an extension of machine learning whereby predictive patterns are learnt and refined by the machine itself, using an algorithm developed from a large example dataset such as a bank of graded retinal images.[154] Multiple levels of increasingly abstract pattern recognition enable the algorithm to develop complex neural networks that are highly accurate, require minimal engineering and can match expert human performance as shown recently with diabetic retinopathy.[155]

Recent work in this field has used retinal photographs and clinical data from >280,000 patients to train an algorithm to predict a range of CVD risk factors from two separate banks of retinal photographs totalling ~13,000 patients.[156] The algorithm displayed impressive accuracy: gender (AUC 0.97), smoking status (AUC 0.71), age (mean absolute error ~3 years) and systolic BP (mean absolute error ~11 mmHg).[156] For predicting incident CVD risk, the algorithm offered little improvement over a conventional CVD risk-scoring tool but encouragingly showed similar power. More recently, one of the authors of this review (PAK), co-led the development of a deep learning algorithm that allows triage and diagnosis of the commonest sight-threatening retinal diseases from OCT scans.[157] On a large retrospective dataset, this algorithm demonstrated diagnostic accuracy equivalent to that of specialist ophthalmologists. Prospective clinical trials of this algorithm are now planned. Importantly, the algorithm also creates an intermediate representation of the retinal anatomy/pathology and thus addresses, in part, the concerns raised by clinicians regarding artificial intelligence systems which can be often perceived as 'black boxes' dictating clinical care. This approach will likely be readily transferable from ophthalmic to systemic disease, offering novel insights and generating new hypotheses.

Challenges

The real-world utility of the retinal vascular metrics for CVD risk profiling over currently available tools is uncertain. Well-designed studies show that such metrics can offer a small benefit (~10%) over current tools in identifying high-risk patients.[66, 81, 89] Whether this is sufficient for integration into clinical practice is not known nor is how best to act on them. The high fidelity and granular nature of OCT data may yield novel metrics that extend risk stratification beyond what retinal photography has achieved. Pre-clinical and clinical studies with longitudinal imaging and data-linkage, currently available in only a few countries, will be required to confirm this. However, the highly competitive commercial interest in retinal OCT has led to emergence of several devices each with unique retinal layer segmentation algorithms and so metrics are not interchangeable across devices.[158, 159] Finally, maximising the yield of data from complex OCT and OCT-A images will require similar advancement and investment in imaging analysis methodology which, given the advent of artificial intelligence in healthcare, is an exciting field in itself.

Conclusions

The eye offers a well-defined target organ whose microvessel network is homologous to that in kidney in both health and disease. The chorioretinal microvasculature can now be precisely mapped, measured and tracked. Quantitative vessel analysis of retinal photographs has provided a strong rationale for the eye to report and stratify CVD risk but this is yet to transition into clinical practice. Novel modalities such as OCT have undergone rapid clinical expansion and have shown potential in detecting microvascular changes that are associated with surrogate markers of increased renal and CVD risk. Advances in data analysis and machine learning may soon enable clinicians to generate individualised chorioretinal risk scores to identify patients at risk of adverse outcomes based on precise, segmented OCT

metrics. The advancement of multimodal functional retinal imaging has brought the previously distant goal of non-invasive functional microvascular assessment into sharp focus and the near term.

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Table legends

Table 1. *Retinal vascular metrics from retinal photography*

Table 2. *Retinal vascular metrics to predict incident or progressive chronic kidney disease*

BP – blood pressure, AVR – arteriole-to-venule ratio, OR – odds ratio, CRAE – central retinal arteriolar equivalents, CRVE – central retinal venular equivalents, RR – risk ratio, IFG – impaired fasting glucose, HR – hazard ratio, eGFR – estimated glomerular filtration rate, CKD – chronic kidney disease, *Df* - fractal dimension, ESRD – end stage renal disease. All values are mean.

Table 3. *Chortioretinal layer metrics from optical coherence tomography*

Table 4. *Haemodialysis and OCT metrics.* Studies examining OCT metrics before and after a haemodialysis session in patients on long-term renal replacement therapy. ↑ denotes increase, ↓ denotes decrease. UF – achieved ultrafiltration volume, BP – blood pressure, IOP – intraocular pressure, RNFL – retinal nerve fibre layer, EDI – enhanced depth imaging, L – litres, SBP – systolic blood pressure, DBP – diastolic blood pressure. All values are mean.

Table 1.

Metric	Derivation	Interpretation	Strengths	Weaknesses
Central retinal arteriolar equivalents (CRAE)	Widths of reflective erythrocyte column within vessel lumen from six largest arterioles located in a zone 0.5-1 disc diameters from optic disc margin	Summarised surrogate measure of internal arteriolar widths that reflect narrowing or widening	Provides insight into disease affecting arterioles Relatively easy to obtain and automate	Summarised rather than absolute values Potential for magnification and positioning errors Values are not true vessel widths nor cross-sectional area which may be more relevant to disease
Central retinal venular equivalents (CRVE)	Widths of reflective erythrocyte column within vessel lumen from six largest venules located in a zone 0.5-1 disc diameters from optic disc margin	Summarised surrogate measure of internal venular width that reflect narrowing or widening	Provides insight into disease affecting venules Relatively easy to obtain and automate	Summarised rather than absolute values Potential for magnification and positioning errors Values not true vessel widths nor cross-sectional area which may be more relevant to disease
Arteriole-to-venule ratio (AVR)	Ratio of CRAE to CVRE	Changes usually indicative of generalised arteriolar narrowing	Avoids magnification errors Dimensionless	Provides little insight to underlying pathophysiology If used alone, can lead to incorrect inferences: both CRVE and CRAE narrow with increasing BP producing a normal AVR masking any association.
Fractal dimensions (*Df*)	Images are binarised and vessel maps are broken up into short segments (‘skeletonisation’). Entire image divided into boxes and those containing a vessel segment are counted. Process is repeated with different box sizes. The number of boxes with vessel segments is plotted against the total number of boxes in the image.	Index of vessel network spatial occupancy (complexity). Reduced (sparse) or increased (dense) complexity relative to health or within a cohort reflect suboptimal vascular network geometry.	Based on robust models of optimality of vascular branching May be more sensitive than calibres in reflecting microvascular disease in other organs beds	Less widely studied than calibres Simplifies 3-dimensional vascular networks into 2-dimensional skeletonised maps

Table 2.

Study	Country	n	Population Mean age	Retinal metric	Clinical outcome	Hypertension	Mean BP	Diabetes	Index serum creatinine or eGFR	Follow up	Results
Wong[59] 2004 Prospective, Population - based cohort	US	10,056	White and African-American adults, eGFR >60mls/min 60 years	AVR	Incident renal dysfunction: rise in serum creatinine by ≥35µmol/L or hospital admission/death coded for renal disease	50%	127/70 mmHg	22%	80µmol/L	6 years	3% developed CKD Smallest AVR associated with greater change in serum creatinine (4 vs 2µmol/L)
Wong[62] 2004 Prospective, Population - based cohort	US	557	Type 1 diabetics eGFR >90mls/min, proteinuria <0.3g/L 31 years	CRAE CRVE	Incident renal insufficiency: eGFR <60mls/min Incident gross proteinuria: >0.3g/L	No data	120/76 mmHg	100%	No data	16 years	20% developed CKD 33% developed proteinuria Widest CRVE quartile associated with increased incidence of CKD and proteinuria CKD: adjusted RR 1.5(1.05-2.2) Proteinuria: adjusted RR 1.5(1.2-2) No association for CRAE
Edwards[63] 2004 Prospective, Population - based cohort	US	1,394	Adults aged >65 years 78 years	AVR	Change in serum creatinine Decline in renal function: increase in serum creatinine by ≥27µmol/L and fall in eGFR by ≥20%	57%	131/67 mmHg	17%	89µmol/L 70mls/min	4 years	4-5% developed a significant increase in serum creatine or fall in eGFR AVR showed no associations with changes in renal function Retinopathy associated with greater risk of decline in renal function: Adjusted OR 2.8-3.2 vs. no retinopathy
Sabanayagam[64] 2011 Prospective, Population - based cohort	US	3,199	White adults with eGFR >60mls/min 59 years	CRAE CRVE	Incident CKD: eGFR <60mls/min and 25% decrease from baseline	45%	130/78 mmHg	9%	85mls/min	15 years	5% developed CKD No association CRAE or CRVE with incident CKD No association with eGFR and incident CRAE narrowing or CRVE widening
Yau[65] 2011	US	4,594	Multi-ethnic adults, eGFR	CRAE CRVE	Incident CKD: eGFR <60mls/min	40%	127/71 mmHg	11%	76mls/min	4.8 years	5% developed CKD

Prospective, Population - based cohort			>60mls/min 64 years								Narrowest CRAE tertile associated with incident CKD in **white patients only:** adjusted HR 1.78 (1.01-3.1) vs. widest; increased to 2.95 when analysing those without hypertension or diabetes No association with CRVE
Baumann[66] 2014 Prospective	Germany	141	Adults, CKD stage 2-4 61 years	CRAE	Progression of CKD: 50% decline in eGFR or start of RRT	No data	137/76 mmHg	46%	48mls/min	3.9 years	17% had progression in CKD Narrowest CRAE tertile associated with progression in CKD: adjusted OR 3 (1.2-7.5) *vs.* widest CRAE Narrowest CRAE in presence of albuminuria associated with 10-fold increased risk of CKD progression, compared to 3-fold risk seen with narrow CRAE or albuminuria alone
Grunwald[67] 2014 Prospective, Population - based cohort	US	1852	Adults, eGFR 20-70mls/min 62 years	AVR CRAE CRVE	Progression in CKD: ESRD/RRT, Change in eGFR slope	90%	130/80 mmHg	47%	40mls/min	2.3 years	8% developed ESRD eGFR decline 0.53ml/min Greater AVR associated with ESRD and steeper eGFR decline: adjusted HR 3.1 (1.5-6.4). No associations with CRAE and CRVE
Yip[68] 2015 Prospective, Population - based cohort	Singapore	5763	Malay adults 55 years	AVR CRAE CRVE *Df*	Incident ESRD: defined by start of renal replacement therapy	55%	140/70 mmHg	34%	77mls/min	4.3 years	0.4% developed ESRD No associations for vascular metrics and risk of ESRD in adjusted analyses Retinopathy predicted ESRD.
Yip[69] 2017 Prospective, Population - based cohort	Singapore	1256	Malay adults 56 years	CRAE CRVE Tortuosity *Df* Branching angles	Incident CKD: eGFR <60mls/min	58%	150/80 mmHg	25%	80mls/min	6 years	6% developed incident CKD Narrower CRAE associated with incident CKD: adjusted HR 1.3 (1-1.78) as continuous variable. Widest CRVE tertile associated with incident CKD: adjusted HR 2.4 (1.1-5.9) *vs.* narrowest No other vascular metrics associated with incident CKD

McKay[70] 2018 Prospective, Population - based cohort	Scotland	1068	Adults eGFR ≥60mls/min 63 years	CRAE CRVE Tortuosity *Df* Branching angles	Change in eGFR: 'Progressors': eGFR <60mls or ≥15% decline 'Non-progressors': <10% decline	No data	138/77 mmHg	100%	94mls/min	3 years	31% had 'progressive' CKD No baseline retinal metric predicted progression of CKD in unadjusted or adjusted analyses.

Table 3.

Metric	Derivation	Interpretation	Strengths	Weaknesses
Retinal thickness	Calculated from the number of A-scan pixels between the internal limiting and Bruch's membranes. Sequential A-scans in horizontal or vertical plane generate a two-dimensional thickness profile across the retina, the B-scan	Average thickness of peripheral and central retinal subfields Thinning predominantly reflects neuronal loss. May also reflect intra-retinal capillary rarefaction Thickening reflects accumulation of oedema, vascular exudates or cellular debris	Can allow detection of differential patterns of retinopathy: global thinning *vs*. predominantly central or peripheral subfield thinning Easy to obtain, automated and highly reproducible Segmentation of retinal sublayers can provide novel insight into pathogenesis such ganglion cell layer	Layer and subfield boundaries not standardised across devices Overall thickness may miss sublayer thinning or thickening Cannot differentiate between neuronal or vascular structures
Macular volume	Calculated as product of retinal thickness and scan area. The scan area can be subdivided into the Early Treatment of Retinopathy in Diabetes Study (ETRDS) map of 6mm, 3mm and 1mm concentric rings centred on the fovea, producing 9 subfields	Global and regional volumes of key region for vision Thinning predominantly reflects neuronal loss. May also reflect intra-retinal capillary rarefaction. Thickening reflects accumulation of oedema, vascular exudates or cellular debris	Can allow early detection and tracking of differential patterns of maculopathy Segmentation of sublayers can provide novel insight into pathogenesis Easy to obtain, automated and highly reproducible	Accuracy dependent on total number of stacked horizontal B-scans within scan area Layer boundaries and B-scan protocols not standardised across devices
Retinal nerve fibre layer thickness	Calculated from the number of A-scan pixels between the internal limiting membrane and ganglion cell layer Sequential A-scans in horizontal, vertical plane or circular plane centred on the optic disc generate a thickness profile	Global and regional assessment of ganglion cell (GC) axon number Thinning reflects GC axonal loss Thickening reflects axonal oedema seen in inflammation, ischaemia or intracranial hypertension	Specific biomarker of optic neuropathy and wider central neurological disease Allows earlier detection and tracking of differential patterns of neuropathy. Easy to obtain, automated and highly reproducible Standardized normative range available for glaucoma and neurological disease	Blood vessels (and glial cells) within RNFL are included in measurement so not truly representative of GC axon population Segmentation errors can occur Susceptible to confounding by optical biometrics such as axial length, disc size, disc-fovea angle
Choroidal thickness	No precise definition or standard methodology. Calculated from the number of A-scan pixels from Bruch's membrane to choroidoscleral interface. Often manually measured at several discrete locations Newer devices provide automatic segmentation to automatically calculate regional choroidal thickness and volume in a manner similar to that for retinal thickness and volume.	Coarse measure of a dense vascular layer containing arterioles, capillaries, venules and veins. Thinning reflects vascular changes including reduced blood flow, vasoconstriction or rarefaction. Contribution of non-vascular components unclear. Thickening may due to increased blood flow, vasodilatation or oedema. Contribution of non-vascular components unclear.	Easy to obtain, increasingly automated and reproducible Assess critical vascular supply to retina and reveals new insight into macular disease	Inaccessible location continues to limit comprehensive vascular assessment Does not differentiate between vascular and non-vascular structures Susceptible to confounding by optical biometrics such as axial length No standardized normative range

Author	Year	Country	Device	*n*	Age	Proportion with diabetes	Dialysis vintage	UF volume	Δ weight	Δ BP	Δ IOP	Δ retinal thickness	Δ choroidal thickness	Δ RNFL	Δ vessel density

Table 4.

Shin[114]	2019	South Korea	DRI Triton	32	56 years	~66%	6 years	3L	↓ 2.6kg	↓ SBP ~12mmHg	No change	-	↓ ~5%	-	-
Shin[115]	2018	South Korea	DRI OCT1 Atlantis	29	56 years	~52%	5.8 years	3L	↓ 2.7g	↓ SBP ~10mmHg	No change	No change	↓ ~7%	-	↓ ~3%
Zhang[116]	2018	China	AngioVue	77	53 years	~50%	4.5 years	2.5L	-	↓ SBP ~7mmHg	No change	↓ ~2%	No change	-	↓ ~3%
Chen[117]	2018	China	Cirrus HD	90	58 years	~13%	5.8 years	-	-	↓ SBP ~10mmHg ↓ DBP ~7mmHg	No change	No change	↓ ~12%	↑ ~3%	-
Chang[118]	2017	South Korea	Spectralis with EDI	54	60 years	~60%	5 years	-	↓ 2.3kg	↓ SBP ~15mmHg ↓ DBP ~4mmHg	↓ ~10%	-	↓ ~10%	No change	-
Ishibazawa[119]	2015	Japan	RetinaScan No EDI	77	67 years	~50%	4.7 years	2.5L	↓ 2.2kg	↓ SBP ~14mmHg ↓ DBP ~4mmHg	No change	No change	↓ ~10%	-	-
Jung[120]	2014	South Korea	Spectralis No EDI	19	51 years	~50%	3.5 years	-	↓ 2.1kg	↓ SBP ~16mmHg	No change	-	↑ ~5%	-	-
Yang[121]	2013	South Korea	Spectralis with EDI	34	58 years	~25%	6 years	-	↓ 2.8 kg	No change	↓ ~10%	No change	↓ ~6%	No change	-
Ulas[122]	2013	Turkey	Spectralis with EDI	21	61 years	-	2.4 years	3L	-	No change	No change	No change	↓ ~10%	-	-
Jung[123]	2013	South Korea	Spectralis No EDI	30	54 years	~40%	4.3 years	-	↓ 1.9kg	↓ SBP ~17mmHg ↓ DBP ~7mmHg	↓ ~15%	↓ ~2%	-	-	-
Theodossiadis[124]	2011	Greece	OCT3 Stratus	72	62 years	100%	2.8 years	-	↓ 2.5kg	No change	-	↓ ~4%	-	-	-
Demir[125]	2008	Turkey	OCT3 Stratus	36	41 years	-	3.5 years	-	-	-	-	-	-	No change	-

Figure legends

Figure 1. ***Initiation and consequences of microvascular disease.***[5]

Light blue arrows show additional association/contribution between insults. *Dark blue arrows* indicate sequence of events leading to development and progression of end-organ dysfunction. GFR: glomerular filtration rate; SVD: small vessel disease; CAD: coronary artery disease; LVH: left ventricular hypertrophy.

Figure 2. ***The eye as a window to the kidney***

The microcirculation of the eye is characterised by multiple capillary networks which, although arranged in close proximity, have striking structural and functional differences. This is also true of the renal microcirculation.

A. *Upper panel* – cross-sectional diagram of glomerular capillary

Lower panel – cortico-medullary microcirculation organisation, oxygen gradients and actions of renal-angiotensin-aldosterone and endothelin systems

B. *Upper panel* – cross-sectional diagram of choroidal capillary

Lower panel – chorioretinal microcirculation organisation, oxygen gradients and actions of renal-angiotensin-aldosterone and endothelin systems

pO_2 – partial pressure of oxygen; ON – optic nerve; RNFL – retinal nerve fibre layer; CRA – central retinal artery; CRV – central retinal vein; RAAS – renin-angiotensin-aldosterone system; ET – endothelin system; AT_1R – angiotensin II type 1 receptors; ET_AR – endothelin type A receptor; ET_BR – endothelin type B receptor.

Figure 3. ***Retinal vascular network geometric indices***

Retinal photographs (left panels) of the left eye using Canon CR-1 fundus camera with a field of view of 45° from a healthy volunteer (**A**) and patient with CKD (**B**). Arterial and venous

branches are binarised and segmented (middle panels) before being transformed into vessel (arterial) skeleton maps (right panels) for fractal dimension (*Df*) analyses. The vessel segmentation and skeleton maps demonstrate retinal vessel rarefaction in CKD compared to health that is not evident from the standard retinal photographs; health Df $_{arteries}$ = 1.47; CKD *Df* $_{arteries}$ = 1.18. Retinal photographs and segmentation images used under Creative Commons licence from https://www5.cs.fau.de/research/data/fundus-images/. Vessel skeleton map images kindly provided by Stephen Hogg (VAMPIRE® group, University of Dundee, United Kingdom).

Figure 4. ***Deep imaging with OCT***

Right eye *en-face* confocal scanning laser ophthalmoscope (CLSO, left panels) and OCT (right panels) images using the Heidelberg SPECTRALIS® Spectral-Domain OCT machine (SD-OCT, Heidelberg Engineering, Heidelberg, Germany). in health (**A**, **B** & **C**) and CKD (**D**) with enhanced depth imaging (**C** & **D**) Scale bars: 200 μm.

A. SD-OCT enables the identification of specific cell layers within the retina in high resolution. *Left panel* shows an *en face* CLSO image centred over the macula. Green line represents level and direction of cross section of corresponding OCT image running from left to right. *Right panel* is an OCT image demonstrating individual layers within the retina. Retinal thickness is defined as the area bounded by internal limiting membrane (ILM) and Bruch's membrane (BM).

B. *Left panel* is a CLSO image centred over the optic nerve head with line of cross-section (green) circled around the peri-papillary region. The dark blue line defines the distance from optic disc to fovea. *Right panel* is an OCT image demonstrating retinal thickness from the circular cross-section around the optic nerve head in the left image. The green line running

from left to right corresponds to the direction of cross-section of the green circle in left panel. Retinal nerve fibre layer thickness as defined as the area bordered by red and cyan lines.

C. CSLO (*left panel*) and OCT with Enhanced Depth Imaging (EDI, *right panel*) in a **healthy subject**. EDI enables identification of deeper structures including the highly vascularised choroid. We measure choroidal thickness at 3 locations: I = 2 mm nasal to the fovea, II = subfoveal, III = 2 mm temporal to the fovea. The corresponding locations on the macula are indicated by yellow arrows.

D. CLSO (*left panel*) and OCT with EDI (*right panel*) in an age-, sex-matched **subject with CKD** demonstrating comparative thinning of the choroid at all 3 locations. The corresponding locations on the macula are indicated by yellow arrows.

Figure 5. ***OCT angiography in health and chronic kidney disease***

Right eye *en face* OCT angiograms centred on the macula using the AngioVue® Imaging System (Optovue, Inc., Freemont, California) in a healthy volunteer (**A**) and an age- and sex-matched patient with proteinuric chronic kidney disease (CKD, **B**).

Left and middle panels: Peri-macular superficial and deep capillary plexuses with the foveal avascular zone (FAZ) represented by central black circular region. Compared to health, a wider FAZ and a disorganised branching pattern are evident in the CKD patient and are suggestive of rarefaction and microvascular damage.

Right panels: Peri-macular superficial retinal vessels and the foveal avascular zone with colour map overlays of software-calculated vessel density. Red denotes high vessel density; green denotes moderate vessel density; blue/navy denotes low vessel density. There is fewer

regions with high / moderate vessel density (red/green regions) CKD compared to health. Note these images are not from the same subjects as the left and middle panels.

Supplementary material

Supplementary figure legends

Supplementary figure 1. ***Alport syndrome-associated retinopathy***.

Ultra-wide field scanning laser ophthalmoscope photograph of left eye from a male patient with end-stage renal disease secondary to X-linked Alport syndrome. The characteristic dot-and-fleck retinopathy is evident as green peri-macular deposits in the centre of the image. There is also associated loss of foveal reflex due to macular thinning. The presence of Alport-associated retinopathy can help diagnosis, suggest inheritance patterns, identify those at risk of progressive CKD and differentiate from mimics such as thin GBM disease which has no associated retinal features.[14, 15]

Supplementary figure 2. ***Retinal photography and fundus retinal calibre assessment***

A. Right eye digital retinal photograph using Canon CR-1 fundus camera with a field of view of 45°.

B. Right eye scanning laser ophthalmoscope image centred over optic disc using the Heidelberg SPECTRALIS® Spectral-Domain OCT machine (SD-OCT, Heidelberg Engineering, Heidelberg, Germany). The centre of the optic disc is manually determined. A standard set of concentric circular measurement zones commonly used in the analysis of fundus camera images is mapped from this point as shown. Zone 'A' is the area 0.5 to 1 optic disc diameters away from the centre as bounded by the white dotted lines. The vessel analysis software VAMPIRE® (University of Dundee, United Kingdom) selects the six widest arterioles (red) and venules (blue) crossing zone A to calculate CRAE, CRVE and AVR.

Supplementary figure 3. ***Retinal optical coherence tomography***.

Blue lines represent fibre paths. *Red lines* represent optical paths. *Green lines* represent signal paths. Low coherence light (typically ~800 nm wavelength) is split into a sample beam and reference beam. The sample beam is shone onto the retina and reflected back as 'light echoes'. The reference beam is directed to a mirror positioned at a known distance from the light source. A range of 'sample' light echoes each return at different times depending on the distance of the reflecting tissue from the light source. Returning 'sample' and 'reference' light echoes are re-united and directed to a photodetector. An interference signal is detected when the time delays between the 'sample' and 'reference' light echoes is small, *i.e*. the distance of reference mirror matches the distance of the reflecting tissue. Moving the reference mirror (positions A to C) alters the distance the reference beam/echo must travel and thus changes the time delay between reference and sample echoes. At each new reference mirror distance, the reference echo time delay will closely match a different sample echo time delay, generating a new interference signal corresponding to a deeper/shallower reflecting tissue layer. Sequential movement of reference mirror allows the construction of a single interference depth profile: the A-scan. A-scans obtain depth profiles along the z-axis. The lateral scanning mirror is rotated through positions 1 to 4 along the x–axis to obtain sequential adjacent A-scans which are used to generate a single horizontal B-scan. The vertical scanning mirror is elevated/lowered along the y-axis to obtain horizontal B-scans at multiple levels. B-scans can then be stacked to create a volume scan (**Supplementary videos 1 and 2**).

Supplementary figure 4. ***Retinal layer segmentation by OCT.*** Close up of horizontal line OCT scan through the macula of the right eye with automated segmentation of individual retinal layers. RNFL – retinal nerve fibre layer, GCL – ganglion cell layer, IPL – inner plexiform layer, INL – inner nuclear layer, OPL – outer plexiform layer, ONL – outer nuclear

layer, PR – photoreceptor layer, RPE – retinal pigment epithelium, BM – Bruch's membrane, CH – choroid.

Supplementary figure 5. ***OCT angiography***

Right eye 3 x 3mm OCT angiograms *en face* (left panels) and in cross-section (right panels), centred on the macula, using the AngioVue® Imaging System (Optovue, Inc., Freemont, California).

A. *Left panel:* The peri-macular superficial capillary plexus and the foveal avascular zone. *Right panel:* OCT image showing the level and boundaries of retinal layer segmentation the corresponds to *en face* angiogram. Red and green lines define the upper and lower limits respectively, of the segmentation band.

B. *Left panel:* The peri-macular deep capillary plexus and the foveal avascular zone. *Right panel:* OCT image showing the level and boundaries of deeper retinal layer segmentation the corresponds to *en face* angiogram. Red and green lines define the upper and lower limits respectively, of the segmentation band.

C. *Left panel:* The outer (deepest) retinal layers are relatively avascular as shown and is thus dependent on passive oxygenation from the deeper choroidal circulation. *Right panel:* OCT image showing the level and boundaries of outer retinal layer segmentation the corresponds to *en face* angiogram. Red lines define the upper and lower limits of the segmentation band.

D. *Left panel:* The subfoveal choriocapillaris is a dense mesh of capillaries underneath the pigment epithelium. Despite advances in OCT-A, imaging discrete vessels here remains challenging. *Right panel:* OCT image showing the level and boundaries of choriocapillaris

segmentation the corresponds to *en face* angiogram. Red and green lines define the upper and lower limits respectively, of the segmentation band.

Supplementary video legends

Supplementary video 1. ***OCT in health***

Video of a 3-dimensional rendered macular volume OCT scan of right eye from a healthy volunteer.

Supplementary video 2. ***OCT in CKD***

Video of a 3-dimensional rendered macular volume OCT scan of right eye from an age- and sex-matched subject with CKD. Note marked thinning of choroidal vascular lying underneath the retina.

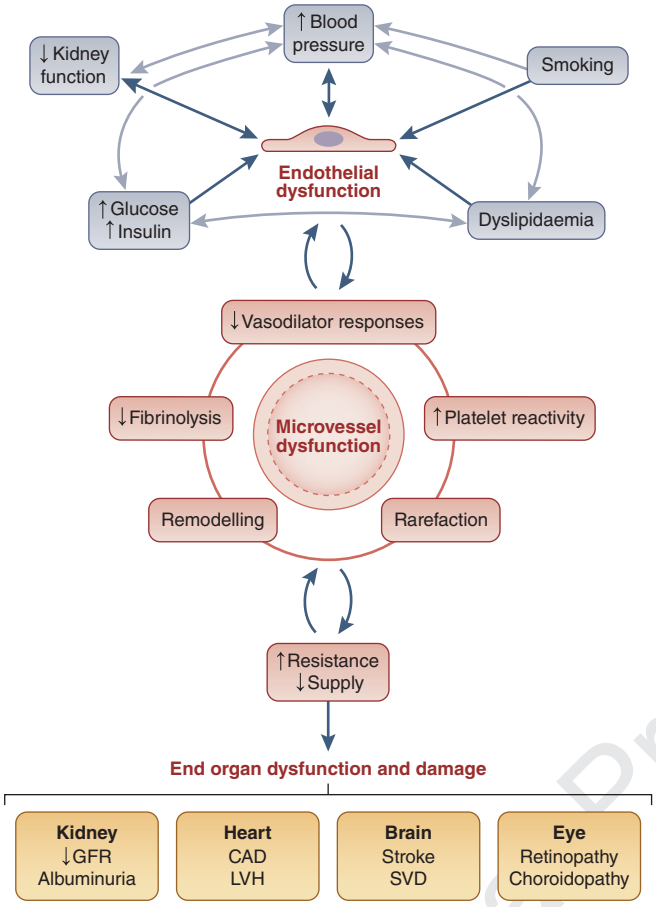
Supplementary video 3. ***OCT angiography of macula in health***

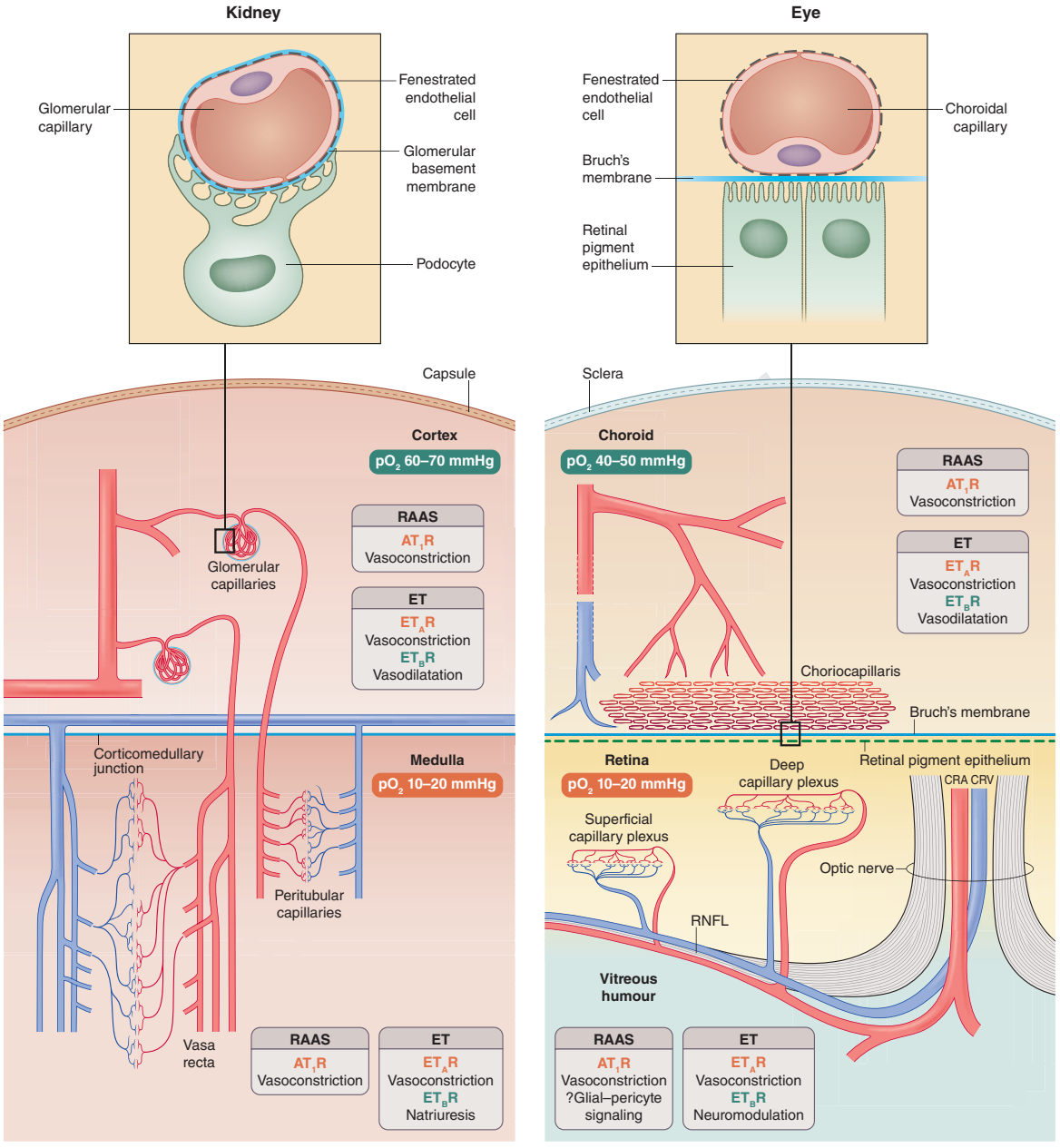
Video of sequential 'layer by layer' OCT angiograms centred on macula of right eye from a healthy volunteer. The track bar on the left-hand side relates colour-coded anatomic localisation of retinal regions that correspond to the angiograms shown in main panel.

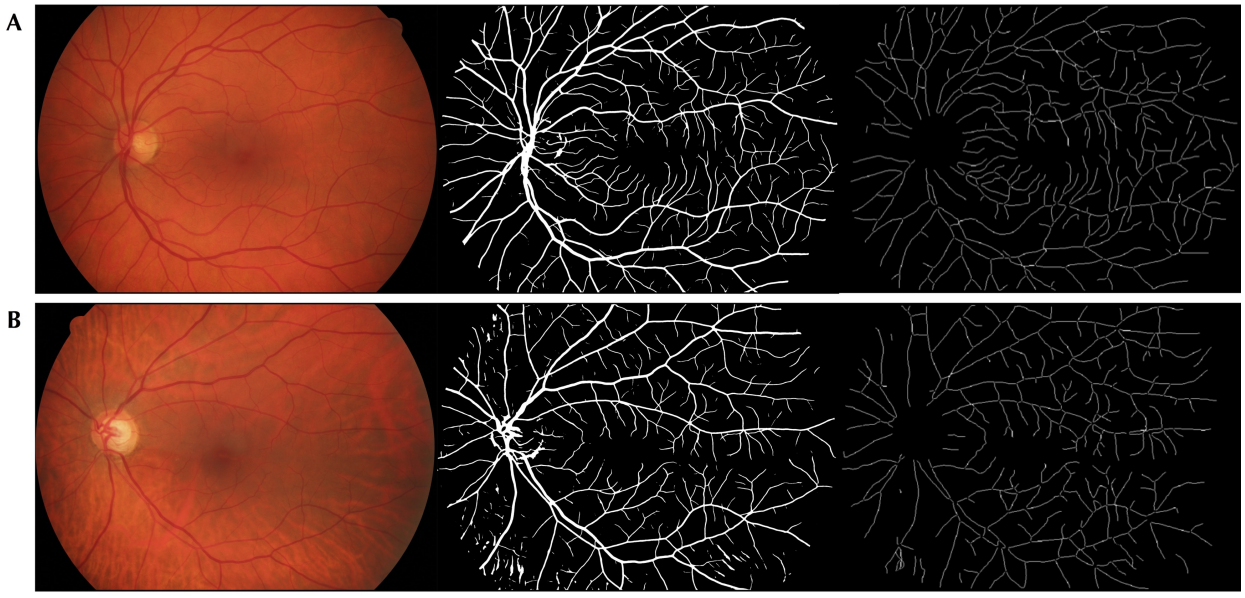
Supplementary video 4. ***OCT angiography of optic disc in health***

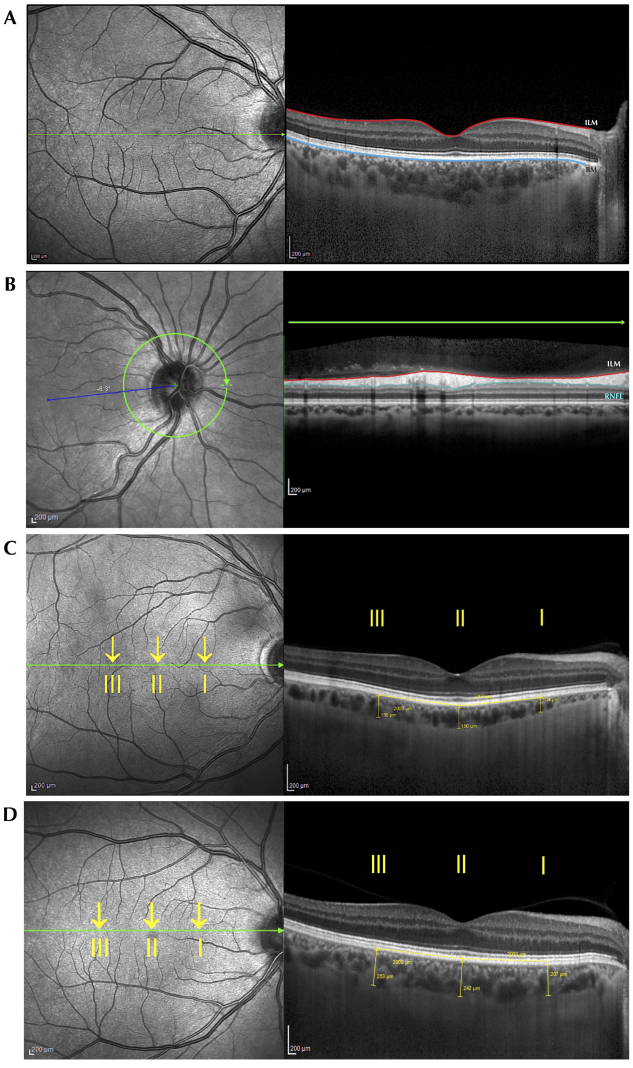
Video of sequential 'layer by layer' OCT angiograms centred on fundus of right eye from a healthy volunteer. The track bar on the left-hand side relates colour-coded anatomic localisation of retinal regions that correspond to the angiograms shown in main panel.

Supplementary information is available on Kidney International's web site




Kidney
Glomerular capillary
Fenestrated endothelial cell
Glomerular basement membrane
Podocyte
Capsule
Cortex
pO_2 60–70 mmHg
RAAS
AT_1R
Vasoconstriction
ET
ET_AR
Vasoconstriction
ET_BR
Vasodilatation
Glomerular capillaries
Corticomedullary junction
Medulla
pO_2 10–20 mmHg
Peritubular capillaries
Vasa recta
RAAS
AT_1R
Vasoconstriction
ET
ET_AR
Vasoconstriction
ET_BR
Natriuresis
Eye
Fenestrated endothelial cell
Choroidal capillary
Bruch's membrane
Retinal pigment epithelium
Sclera
Choroid
pO_2 40–50 mmHg
RAAS
AT_1R
Vasoconstriction
ET
ET_AR
Vasoconstriction
ET_BR
Vasodilatation
Choriocapillaris
Bruch's membrane
Retina
pO_2 10–20 mmHg
Deep capillary plexus
Retinal pigment epithelium
CRA CRV
Superficial capillary plexus
Optic nerve
RNFL
Vitreous humour
RAAS
AT_1R
Vasoconstriction
?Glial–pericyte signaling
ET
ET_AR
Vasoconstriction
ET_BR
Neuromodulation




A
ILM
B
ILM
RNFL
C
III
II
I
D
III
II
I